

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.: 1
Effective Date: August 28, 2012
Sponsor: Ecolab, Inc.
Ecolab Schuman Campus, ESC-F6
655 Lone Oak Drive
Eagan, MN 55121-1560
Test Facility: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121
Protocol Title: Virucidal Efficacy of a Disinfectant for Use on Inanimate
Environmental Surfaces
ATS Labs Protocol Number: ECO01072512.MNV
ATS Labs Project Number: A13932

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INITIALS SC DATE 9/21/12

Modifications to Protocol:

This protocol is amended to include the test substance dilution procedure for each batch of Aqualogic based on the Sponsor's chemical analysis of the test substance on the day of the efficacy testing. Per Sponsor, each batch of test substance will be diluted to the lower certified limit of 660 ppm available chlorine using the following dilution procedures:

Batch 073112-CL1: 356.99g of test substance + 143.01 g of diluent (sterile deionized water).

Batch 080112-CL1: 352.78 g of test substance + 147.22 g of diluent (sterile deionized water).

Note: The weights may vary by +/-0.03 grams. Equivalent dilutions may be made.

Changes to the protocol are acceptable as noted.

Sharon L. Conway
Study Director

8/28/12
Date

(For Laboratory Use Only)
ATS Labs Project # **A13932**

LC 8/21/12

ATS LABS

PROTOCOL

**Virucidal Efficacy of a Disinfectant for Use on
Inanimate Environmental Surfaces**

Virus: Murine Norovirus (MNV-1)

PROTOCOL NUMBER

ECO01072512.MNV

PREPARED FOR

Ecolab, Inc.
Ecolab Schuman Campus, ESC-F6
655 Lone Oak Drive
Eagan, MN 55121-1560

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED BY

Shanen Conway, B.S.
Research Scientist I

DATE

July 25, 2012

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PROPRIETARY INFORMATION

THIS DOCUMENT IS THE PROPERTY OF AND CONTAINS PROPRIETARY INFORMATION OF ATS LABS. NEITHER THIS DOCUMENT, NOR INFORMATION CONTAINED HEREIN IS TO BE REPRODUCED OR DISCLOSED TO OTHERS, IN WHOLE OR IN PART, NOR USED FOR ANY PURPOSE OTHER THAN THE PERFORMANCE OF THIS WORK ON BEHALF OF THE SPONSOR, WITHOUT PRIOR WRITTEN PERMISSION OF ATS LABS.

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Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces

SPONSOR: Ecolab, Inc.
Ecolab Schuman Campus, ESC-F6
655 Lone Oak Drive
Eagan, MN 55121-1560

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE

The purpose of this study is to evaluate the virucidal efficacy of a test substance against Murine Norovirus for registration of a product as a virucide. The test procedure is to simulate the way in which the product is intended to be used. This method is in compliance with the requirements of and may be submitted to, one or more of the following agencies as indicated by the Sponsor: U.S. Environmental Protection Agency (EPA), Health Canada Therapeutic Products Directorate (TPD) and Australian Therapeutic Goods Administration (TGA).

TEST SUBSTANCE CHARACTERIZATION

Test substance characterization as to content, stability, solubility, storage, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized before the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is August 8, 2012. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of August 29, 2012. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, because of failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs nor any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the chosen regulatory agency of its submission concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulatory agencies require that a specific virucidal claim for a disinfectant intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the test substance against the claimed virus. The agency will accept adequate data generated by any appropriate technique in support of a virucidal efficacy claim. This is accomplished by treating the target virus with the disinfectant (test substance) under conditions, which simulate as closely as possible, in the laboratory, the actual conditions under which the disinfectant is designed to be used. For disinfectant products intended for use on hard surfaces (dry, inanimate environmental surfaces), a carrier method is used in the generation of the supporting virological data. The RAW 264.7 macrophage cell line, which supports the growth of the Murine Norovirus, will be used in this study. The experimental design in this protocol meets these requirements.

TEST PRINCIPLE

A film of virus, dried on a glass surface, is exposed to the test substance for a specified exposure time. Following exposure, the virucidal and cytotoxic activities are removed from the virus-test substance mixture, and the mixture is assayed for viral infectivity by an accepted assay method. Appropriate virus, test substance cytotoxicity, and neutralization controls are run concurrently.

STUDY DESIGN

Dried virus films will be prepared in parallel and used as follows:

The appropriate number of films for each batch of test substance assayed per exposure time requested.

The appropriate number of films for virus control titration (titer of virus after drying) per exposure time requested.

At the end of the specified exposure time, resuspended virus-test substance mixtures will be detoxified and made non-virucidal by immediately adding the contents to a Sephadex gel filtration column followed by 10-fold serial dilutions in test medium. Each dilution is inoculated into indicator cell cultures. The resuspended virus control film and each batch of test substance alone will be treated in exactly the same manner. For analysis of infectivity, cultures will be held for the appropriate incubation period at the end of which time cultures will be scored for the presence of the test virus. Cultures will be monitored at that time for cell viability. Uninfected indicator cell cultures will be carried in parallel and similarly monitored. For analysis of cytotoxicity, the viability of cultures inoculated with dilutions of each test and cytotoxicity control will be determined. In addition to the above titrations for infectivity and cytotoxicity, the residual virucidal activity of the test substance after neutralization will be determined by adding a low titer of stock virus to each dilution of the test substance (cytotoxicity control dilutions). The resulting mixtures of dilutions are assayed for infectivity in order to determine the dilution(s) of test substance at which virucidal activity, if any, is retained.

VIRUS

The MNV-1.CW1 strain of Murine Norovirus (MNV-1) to be used for this study was obtained from Washington University, St. Louis, MO. Stock virus is prepared by collecting the supernatant culture fluid from infected culture cells. The cells are disrupted and cell debris removed by centrifugation. The supernatant is removed, aliquoted, and the high titer stock virus may be stored at $\leq -70^{\circ}\text{C}$ until the day of use. On the day of use, an aliquot is removed, thawed, and maintained at a refrigerated temperature until used in the assay. **Note:** If the Sponsor requests an organic soil load challenge, fetal bovine serum (FBS) will be incorporated into the stock virus aliquot. The percent FBS contained in the stock virus aliquot will be adjusted to yield the percent soil load requested.

INDICATOR CELL CULTURES

RAW 264.7 cells, a continuous mouse macrophage cell line originally obtained from Washington University, St. Louis, MO, are propagated by ATS Labs personnel and used as the indicator cell line for this assay. The cells are seeded into multiwell cell culture plates and maintained at $36-38^{\circ}\text{C}$ in a humidified atmosphere of 5-7% CO_2 . The confluency of the cells will be appropriate for the test virus. Alternatively, cells received from the American Type Culture Collection (ATCC), Manassas, VA may be used. The source of the cells will be documented in the raw data and reported.

All cell culture documentation is retained for the cell cultures used in this assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

TEST MEDIUM

The test medium used for this assay is Complete 2X MEM.

PREPARATION OF TEST SUBSTANCE

The dilution of test substance(s) will be used as recommended by the Sponsor. The product will be pre-equilibrated to the desired test temperature if applicable.

PREPARATION OF VIRUS FILMS

Films of virus will be prepared by spreading 200 μ L of virus inoculum uniformly over the bottom of the appropriate number of 100 X 15 mm sterile glass petri dishes (without touching the sides of the petri dish). The virus will be air-dried at 10°C-30°C until visibly dry (≥ 20 minutes). A calibrated timer will be used for timing the drying. The drying conditions (temperature and humidity) will be appropriate for the test virus for the purpose of obtaining maximum survival following drying. The actual drying conditions, drying time and calibrated timer used will be clearly documented.

For U. S. EPA, Australian TGA, and internal/other use only, one dried virus film per batch of test substance will be assayed unless otherwise requested. For Health Canada TPD, five dried virus films per batch of test substance will be assayed unless otherwise requested. If multiple regulatory agencies are chosen, the greater number of virus films will be assayed.

TEST METHOD

Preparation of Sephadex Gel Filtration Columns

To reduce the cytotoxic level of the virus-test substance mixture prior to assay of virus, and/or to reduce the virucidal level of the test substance, virus is separated from the test substance by filtration through Sephadex gel. The type of Sephadex used will be specified in the final report. On the day of testing, Sephadex columns are prepared by centrifuging the prepared Sephadex gel in sterile syringes for three minutes to clear the void volume. The columns are now ready to be used in the assay.

Input Virus Control

On the day of testing, the stock virus utilized in the assay will be titered by 10-fold serial dilution and assayed for infectivity to determine the starting titer of the virus. The results of this control are for informational purposes only.

Treatment of Virus Films with the Test Substance

For each batch of test substance assayed, the appropriate number of dried virus films are individually exposed to a 2.0 mL aliquot of the use dilution of the test substance (liquid products), or to the amount of spray released under use conditions (spray products) and held covered for the specified exposure time(s) and temperature. A calibrated timer will be used for timing the exposure. The actual temperature will be recorded. Just prior to the end of the exposure time, the plates are individually scraped with a cell scraper to resuspend the contents and at the end of the exposure time the virus-test substance mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger in order to detoxify the mixture. The filtrate (10^{-1} dilution) is then titered by serial dilution and assayed for infectivity and/or cytotoxicity. To further aid in the removing of the cytotoxic effects of the test substance to the indicator cell cultures, individual dilutions may be passed through additional individual Sephadex columns.

Treatment of Dried Virus Control Film

The appropriate number of virus films are prepared as described previously for each exposure time assayed. The virus control films are run in parallel to the test virus but a 2.0 mL aliquot of test medium is added in lieu of the test substance. The virus control films are held covered and exposed to the test medium for the same exposure time and at the same exposure temperature as the test films are exposed to the test substance. A calibrated timer will be used for timing the exposure and the actual temperature will be recorded. Just prior to the end of the exposure time, the virus films are individually scraped as previously described and at the end of the exposure time the mixtures are immediately passed through individual Sephadex columns utilizing the syringe plunger. The filtrate (10^{-1} dilution) is then titered by serial dilution and assayed for infectivity. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the virus control will be passed through additional individual Sephadex columns.

Cytotoxicity Control

A 2.0 mL aliquot of each batch of test substance (liquid products) or the amount of the test substance recovered when sprayed onto a sterile petri dish (spray products), is filtered through a Sephadex column utilizing the syringe plunger and the filtrate is diluted serially in medium and inoculated into cell cultures for assay of cytotoxicity concurrently with the virus control and test substance-treated virus samples. For spray products, the cytotoxicity control will be held covered for the longest requested exposure time at the requested exposure temperature. A calibrated timer will be used for timing the exposure. If additional Sephadex columns were used to further reduce the cytotoxic effects in the test substance assay, the same dilutions of the cytotoxicity control will be passed through additional individual Sephadex columns.

Assay of Non-Virucidal Level of Test Substance (Neutralization Control)

Each dilution of the neutralized test substance (cytotoxicity control dilutions) will be challenged with an aliquot of low titer stock virus to determine the dilution(s) of test substance at which virucidal activity, if any, is retained. Dilutions that show virucidal activity will not be considered in determining reduction of the virus by the test substance.

Using the cytotoxicity control dilutions prepared above, an additional set of indicator cell cultures will be inoculated with a 250 µL aliquot of each dilution in quadruplicate. The 10⁻¹ dilution may be inoculated in duplicate or triplicate, if insufficient volume for quadruplicate. A 100 µL aliquot of low titer stock virus will be inoculated into each cell culture well and the indicator cell cultures will be incubated along with the test and virus control plates.

Infectivity Assays

The RAW 264.7 macrophage cell line will be used as the indicator cell line in the infectivity assays. Cells in multiwell culture dishes will be inoculated in quadruplicate with a 250 µL aliquot of the dilutions prepared from the test and control groups. The input virus control will be inoculated in duplicate. The 10⁻¹ dilution for the cytotoxicity and neutralization controls may be inoculated in duplicate or triplicate, if insufficient volume for quadruplicate. Uninfected indicator cell cultures (cell controls) will be inoculated with test medium alone. The inoculum is allowed to adsorb for approximately 60 minutes at room temperature. Following the adsorption time, the inoculum is removed and an aliquot of MNV Overlay Agarose I is inoculated into each well of the cell culture plates. The cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂ for approximately two days. Following incubation, an aliquot of MNV Overlay Agarose II containing neutral red stain is added and the cultures are incubated at 36-38°C in a humidified atmosphere of 5-7% CO₂ for approximately four hours. Following incubation, the cell cultures are visually observed for viral specific plaques. The cultures may be microscopically observed to verify plaques or test substance cytotoxicity.

DATA ANALYSIS

Calculation of Titers

Viral and cytotoxicity titers will be expressed as -log₁₀ of the 50 percent titration endpoint for infectivity (PFU₅₀) or cytotoxicity (TCD₅₀), respectively, as calculated by the method of Spearman Karber.

$$-\text{Log of 1st dilution inoculated} - \left[\left(\left(\frac{\text{Sum of \% mortality at each dilution}}{100} \right) - 0.5 \right) \times (\text{logarithm of dilution}) \right]$$

Calculation of Log Reduction

Dried Virus Control Log PFU₅₀ - Test Substance Log PFU₅₀ = Log Reduction

If multiple dried virus control replicates are performed, the average titer of the replicates will be calculated and the average titer will be used to calculate the log reduction in viral titer of the individual test replicates.

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PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

The specialized virucidal testing section of ATS Labs maintains Standard Operating Procedures (SOPs) relative to virucidal efficacy testing studies. Virucidal efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including virus and cell stocks for purposes of identification, receipt and use of chemical reagents including cell culture medium components, etc. These procedures are designed to document each step of virucidal efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each virucidal efficacy test is assigned a unique Project Number when the Study Director initiates the protocol for the study. This number is used for identification of the test culture plates, etc. during the course of the test. Test culture plates are also labeled with reference to the test virus, experimental start date, and test product. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: N/A

STUDY ACCEPTANCE CRITERIA

Only the applicable acceptance criteria and references for the regulatory agency reviewing the data will be included in the final report.

U.S. EPA Submission

A valid test requires 1) that at least 4 log₁₀ of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. If any of the previous requirements are not met, the test may be repeated under the current protocol number. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

Health Canada TPD Submission

A valid test requires 1) at least a 4-log infectivity be recovered from the dried virus control film beyond the cytotoxic level of the test substance; 2) that the cell controls be negative for infectivity. If any of the previous requirements are not met, the test may be repeated under the current protocol number. **Note:** An efficacious product must demonstrate at least a 3 log₁₀ reduction in viral titer beyond the cytotoxic level of the test substance.

Australian TGA Submission

A valid test requires 1) that at least 4 log₁₀ of infectivity be recovered from the dried virus control film; 2) that when cytotoxicity is evident, at least a 3-log reduction in titer is demonstrated beyond the cytotoxic level; 3) that the cell controls be negative for infectivity. If any of the previous requirements are not met, the test may be repeated under the current protocol number. **Note:** An efficacious product must demonstrate complete inactivation of the virus at all dilutions.

FINAL REPORT

The report will include, but not be limited to, identification of the sample and date received, dates on which the test was initiated and completed, identification of the virus strain used and composition of the inoculum, description of cells, medium and reagents, description of the methods employed, tabulated results, calculated titers for infectivity and cytotoxicity, and a conclusion as it relates to the purpose of the test. A draft report may be requested by the Sponsor. The final report will be prepared once the Sponsor has reviewed the draft report and notified the Study Director to complete the study.

PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

TEST SUBSTANCE RETENTION

Test substance retention shall be the responsibility of the Sponsor. Unused test substance will be discarded following study completion unless otherwise requested.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of the final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study, which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

PROPOSED STATISTICAL METHODS: N/A

REFERENCES

1. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1053 (Current Version).
2. Annual Book of ASTM Standards, Section 11 Water and Environmental Technology Volume 11.05 Pesticides; Environmental Assessment; Hazardous Substances and Oil Spill Response, E 1482 (Current Version).
3. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Public Health Uses of Antimicrobial Agents, March 12, 2012.
4. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces – Efficacy Data Recommendations, March 12, 2012.
5. Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections. Lennette, E. H, Lennette, D.A., and Lennette, E.T. editors. Seventh edition, 1995.
6. Blackwell, J.H., and J.H.S. Chen. 1970. Effects of various germicidal chemicals on HEP-2 cell culture and Herpes simplex virus. J. AOAC 53:1229-1236.
7. Canadian General Standards Board, Minister of Public Works and Government Services, August 1997. Assessment of Efficacy of Antimicrobial Agents for Use on Environmental Surfaces and Medical Devices, CAN/CGSB-2.161-97.
8. Health Canada Therapeutic Products Directorate, October 29, 2007. Guidance Document: Disinfectant Drugs, Health Products and Food Branch.
9. Australian Therapeutic Goods Administration (TGA), February 1998. Guidelines for the Evaluation of Sterilants and Disinfectants.
10. Australian Therapeutic Goods Administration (TGA), February 1998. Therapeutic Goods Order No. 54: Standard for Disinfectants and Sterilants.
11. Australian Therapeutic Goods Administration (TGA), March 1997. Therapeutic Goods Order No. 54A: Amendment to Standard for Disinfectants and Sterilants (TGO 54).
12. Australian Therapeutic Goods Administration (TGA), July 2005. Draft Guidelines for the Evaluation of Household/Commercial and Hospital Grade Disinfectants.
13. Wobus CE, Karst SM, Thackray LB, Chang KO, Sosnovtsev SV, et al. (2004) Replication of *Norovirus* in cell culture reveals a tropism for dendritic cells and macrophages. PLoS Biol 2(12):e432.

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STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Test Substance (Name and Batch Number - exactly as it should appear on final report):

Aquabacter Batches: 073112-CL1 & 080112-CL1

Expiration Date: Proposed dates: 073112-CL1 - 08/31/12 & 080112-CL1 - 09/01/12

Product Description

- ☐ Quaternary ammonia
☐ Iodophor

- ☐ Peracetic acid
☐ Peroxide

- ☒ Sodium hypochlorite
☐ Other

Test Substance Active Concentration (upon submission to ATS Labs): ~~0.086%~~ ~ 0.086% changed for clarity 08/17/12

Storage Conditions

- ☐ Room Temperature

☒ 2-8°C

- ☐ Other

Hazards

- ☐ None known: Use Standard Precautions
☒ Material Safety Data Sheet, Attached for each product
☐ As Follows:

Product Preparation

- ☐ No dilution required, Use as received (RTU)
☒ *Dilution(s) to be tested:

defined as _____ + _____
(example: 1 oz/gallon) (amount of test substance) (amount of diluent)
☒ Deionized Water (Filter or Autoclave Sterilized)
☒ Tap Water (Filter or Autoclave Sterilized)
☐ AOAC Synthetic Hard Water: _____ PPM
☐ Other _____

08/17/12
wrong
lot

08/17/12 The test substance batches will be diluted to the lower certified limit of 600ppm available chlorine. The dilution procedure will be provided by the sponsor the day of efficacy testing following chemical analysis of the test substance.

*Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Virus: Murine Norovirus

Exposure Time: 30 seconds

Exposure Temperature: ☒ Room temperature (to be based on regulatory agency of submission)
☐ Other: _____ °C (please specify range)

Directions for application of aerosol/spray products: 3 spray at a distance of 6-8 inches
☐ Check here if spray instructions are not applicable.

Organic Soil Load

- ☐ 1% fetal bovine serum (minimum level that can be tested)
☒ 5% fetal bovine serum
☐ Other

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REGULATORY AGENCY(S) THAT MAY REVIEW DATA

- ☒ U.S. EPA
☐ Health Canada (Canadian TPD)
☐ Therapeutic Goods Administration (Australian TGA)
☐ Not applicable - For Internal/other use only (Efficacy result will be based on U.S. EPA requirements)

COMPLIANCE

This study will be conducted in compliance with the EPA Good Laboratory Practices Regulations of 40 CFR Part 160 (Federal Register Notice [August 17, 1989]) and in accordance to standard operating procedures.

- ☒ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

- ☒ Approved without modification
☒ Approved with modification

*8/8/12
wrong*
~~Please include Fedats GIP Study Number 1200074CL4 in the final report.~~

~~Also include that the chemical characterization of the test substance was conducted under Ecolab GIP study number 1200074. Also include that~~

PROTOCOL ATTACHMENTS

~~the test substance batches were diluted to the lower Supplemental Information Form Attached - ☐ Yes ☒ No certified limit of 660ppm Available Chlorine for use in efficacy testing.~~

TEST SUBSTANCE SHIPMENT STATUS

- ☐ Has been used in one or more previous studies at ATS Labs.
☐ Has been shipped to ATS Labs (but has not been used in a previous study).

Date shipped to ATS Labs: _____

Sent via overnight delivery? ☐ Yes ☐ No

- ☒ Will be shipped to ATS Labs.

Date of expected receipt at ATS Labs: 8/14/12

- ☐ Sender (if other than Sponsor): _____

Template: 110-5E

- Proprietary Information -

1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

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APPROVAL SIGNATURES

SPONSOR:

NAME: Ms. Laurinda Holen TITLE: Microbiologist
SIGNATURE: *Laurinda Holen* DATE: 8/7/12
PHONE: (651) 795-5974 FAX: (651) 204-7501 EMAIL: laurinda.holen@ecolab.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study:

☐ See Attached

ATS Labs:

NAME: *Shanen Conway*
Study Director
SIGNATURE: *Shanen L. Conway* DATE: 8/20/12
Study Director

Template: 110-5E

- Proprietary Information -

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